United States
Environmental Protection
Agency

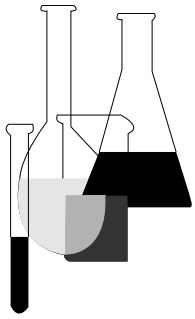
Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–96–253
June 1996

EPA

Health Effects Test Guidelines

OPPTS 870.8320 Oral/Dermal Pharmacokinetics



“Public Draft”

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines’’ or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board*. By modem dial 202–512–1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202–512–0132 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines.’’

OPPTS 870.8320 Oral/dermal pharmacokinetics.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is OPPT 40 CFR 795.228 Oral/Dermal Pharmacokinetics.

(b) **Purpose**. The purpose of these studies is to ascertain whether the pharmacokinetics and metabolism of a chemical substance or mixture (''test substance'') are similar after oral and dermal administration; determine bioavailability of a test substance after oral and dermal administration; and to examine the effects of repeated dosing on the pharmacokinetics and metabolism of the test substance.

(c) **Definitions.** The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definitions also apply to this test guideline.

Bioavailability refers to the rate and relative amount of administered test substance which reaches the systemic circulation.

Metabolism means the study of the sum of the processes by which a particular substance is handled in the body and includes absorption, tissue distribution, biotransformation, and excretion.

Percent absorption means 100× the ratio between total excretion of radioactivity following oral or dermal administration and total excretion following intravenous administration of test substance.

Pharmacokinetics means the study of the rates of absorption, tissue distribution, biotransformation, and excretion.

(d) **Test procedures**—(1) **Animal selection**—(i) **Species.** The rat should be used for pharmacokinetics testing because it has been used extensively for metabolic and toxicological studies. For dermal bioavailability studies, the rat and the miniature pig should be used.

(ii) **Test animals.** For pharmacokinetics testing and dermal studies, adult male and female Sprague-Dawley rats, 7 to 9 weeks of age, should be used. For dermal studies, young adult miniature pigs should also be used. The animals should be purchased from a reputable dealer and should be identified upon arrival at the testing laboratory. The animals should be selected at random for the test groups and any animal showing signs of ill health should not be used. In all studies, unless otherwise specified, each test group should contain at least four animals of each sex for a total of at least eight animals.

(iii) **Animal care**. (A) The animals should be housed in environmentally controlled rooms with at least 10 air changes per hour. The rooms should be maintained at a temperature of 24 ± 2 °C and humidity of 50 ± 20 percent with a 12–h light/dark cycle per day. The animals should be kept in a quarantine facility for at least 7 days prior to use and should be acclimated to the experimental environment for a minimum of 48 h prior to administration of the test substance.

(B) During the acclimatization period, the animals should be housed in suitable cages. All animals should be provided with certified feed and tap water ad libitum. The miniature pig diet should be supplemented with adequate amounts of ascorbic acid in the drinking water.

(2) **Administration of test substance**—(i) **Test substance**. The use of a radioactive test substance is required for all studies. The purity, radioactive and nonradioactive, should be greater than 99 percent. The radioactive and nonradioactive test substances should be chromatographed separately and together to establish purity and identity. If the purity is less than 99 percent or if the chromatograms differ significantly, EPA should be consulted.

(ii) **Dosage and treatment**—(A) **Intravenous**. The low dose of test substance, in an appropriate vehicle, should be administered intravenously to groups of rats and miniature pigs of each sex. If feasible, the same low dose should be used for intravenous, oral, and dermal studies.

(B) **Oral**. Two doses of text substance should be used in the oral study, a low dose, and a high dose. The high dose should induce some overt toxicity, such as weight loss. The low dose should correspond to a no-observed-effect level. The oral dosing should be accomplished by gavage or by administering the encapsulated test substance. If feasible, the same high and low doses should be used for oral and dermal studies.

(C) **Dermal**—(*1*) **Dermal treatment.** For dermal treatment, two doses, comparable to the low and high oral doses, should be dissolved in a suitable vehicle and applied in volumes adequate to deliver comparable doses. The backs of the animals should be lightly shaved with an electric clipper 24 h before treatment. The test substance should be applied to the intact shaven skin (approximately 2 cm^2 for rats and 5 cm^2 for miniature pigs). The dosed areas should be protected with a suitable porous covering which is secured in place, and the animals should be housed separately.

(*2*) **Washing efficacy study.** Before initiation of the dermal absorption studies, an initial washing efficacy experiment should be conducted to assess the removal of the applied low dose of the test substance by washing the exposed skin area with soap and water and an appropriate organic solvent. The low dose should be applied to four rats and four miniature pigs in accordance with paragraph (d)(2)(ii)(C)(*1*) of this guide-

line. After application (5 to 10 min), the treated areas of two rats and two miniature pigs should be washed with soap and water and the treated areas of the remaining rats and pigs should be washed with an appropriate solvent. The amounts of test substance recovered in the washings should be determined to assess efficacy of its removal by washing.

(iii) **Dosing and sampling schedule**—(A) **Rat studies**. After administration of the test substance, each rat should be placed in a metabolic unit to facilitate collection of excreta. For the dermal studies, excreta from the rats should also be collected during the 6–h exposure periods. At the end of each collection period, the metabolic units should be cleaned to recover any excreta that might adhere to them. All studies, except the repeated dosing study, should be terminated at 7 days or after at least 90 percent of the radioactivity has been recovered in the excreta, whichever occurs first.

(*1*) **Intravenous study**. Group A should be dosed once intravenously at the low dose of test substance.

(*2*) **Oral study**. (*i*) Group B should be dosed once orally with the low dose of test substance.

(*ii*) Group C should be dosed once orally with the high dose of test substance.

(*3*) **Dermal studies**. Unless precluded by corrosivity, the test substance should be applied and kept on the skin for a minimum of 6 h. At the time of removal of the porous covering, the treated area should be washed with an appropriate solvent to remove any test substance that may be on the skin surface. Both the covering and the washing should be assayed to recover residual radioactivity. At the termination of the studies, each animal should be sacrificed and the exposed skin area removed. An appropriate section of the skin should be solubilized and assayed for radioactivity to ascertain if the skin acts as a reservoir for the test substance. Studies on the dermal absorption of corrosive test substances should be discussed with EPA prior to initiation.

(*i*) Group D should be dosed once dermally with the low dose of test compound.

(*ii*) Group E should be dosed once dermally with the high dose of the test substance.

(*4*) **Repeated dosing study**. Group F should receive a series of single daily oral low doses of nonradioactive test substance over a period of at least 7 days. At 24 h after the last nonradioactive dose, a single oral low dose of radioactive test substance should be administered. Following dosing with the radioactive substance, the rats should be placed in individual metabolic units as described in paragraph (d)(2)(iii) of this guideline. The

study should be terminated at 7 days after the last dose, or after at least 90 percent of the radioactivity has been recovered in the excreta, whichever occurs first.

(B) **Miniature pig studies**. For all miniature pig studies, the test groups should consist of four young adult animals. After administration of the test substance, each miniature pig should be kept in a metabolic unit to facilitate collection of excreta. At the end of each collection period, the metabolic units are to be cleaned to recover any excreta that might adhere to them. All studies should be terminated at 7 days, or after at least 90 percent of the radioactivity has been recovered in the excreta, whichever occurs first.

(*1*) **Intravenous study.** Group G is to be dosed once intravenously at the low dose of the test substance.

(*2*) **Dermal studies.** Following the experimental guidance described in paragraph (d)(2)(iii)(A)(*3*) of this guideline:

(*i*) Group H should be dosed once dermally with the low dose of test substance.

(*ii*) Group I should be dosed once dermally with the high dose of the test substance.

(3) **Types of studies**—(i) **Pharmacokinetics studies**—(A) **Rat studies**. Groups A through F should be used to determine the kinetics of absorption of the test substance. In the group administered the test substance by intravenous routes, (i.e., Group A), the concentration of radioactivity in blood and excreta should be measured following administration. In groups administered the test substance by the oral and dermal route (i.e., Groups B, C, D, E, and F), the concentration of radioactivity in blood and excreta should be measured at selected time intervals during and following the exposure period.

(B) **Miniature pig studies.** Groups G, H, and I should be used to determine the extent of dermal absorption of the test substance. The amount of radioactivity in excreta should be determined at selected time intervals.

(ii) **Metabolism studies—Rat studies.** Groups A through F should be used to determine the metabolism of the test substance. Urine, feces, and expired air should be collected for identification and quantification of the test substance and metabolites.

(4) **Measurements**—(i) **Pharmacokinetics.** Four animals from each group should be used for these purposes.

(A) **Rat studies**—(*1*) **Bioavailability.** The levels of radioactivity should be determined in whole blood, blood plasma or blood serum at 15 and 30 min and at 1, 2, 8, 24, 48, and 96 h after initiation of dosing.

(*2*) **Extent of absorption.** The total quantities of radioactivity should be determined for excerta collected daily for 7 days or until at least 90 percent of the radioactivity has been recovered in the excreta.

(*3*) **Excretion.** The quantities of radioactivity eliminated in the urine, feces, and expired air should be determined separately at appropriate time intervals. The collection of carbon dioxide may be discontinued when less than one percent of the dose is found to be exhaled as radioactive carbon dioxide in 24 h.

(*4*) **Tissue distribution.** At the termination of each study, the quantities of radioactivity in blood and in various tissues, including bone, brain, fat, gastrointestinal tract, gonads, heart, kidney, liver, lungs, muscle, skin, and residual carcass of each animal should be determined.

(*5*) **Changes in pharmacokinetics.** Results of pharmacokinetics measurements (i.e., bioavailability and extent of absorption, tissue distribution, and excretion) obtained in rats receiving the single low oral dose of the test substance (Groups B and C) should be compared to the corresponding results obtained in rats receiving repeated oral doses of the test substance (Group F).

(B) **Miniature pig studies—Extent of absorption.** The total quantities of radioactivity should be determined for excreta daily for 7 days or until at least 90 percent of the test substance has been excreted.

(ii) **Metabolism.** Four animals from each group should be used for these purposes.

(A) **Rat studies**—(*1*) **Biotransformation.** Appropriate qualitative and quantitative methods should be used to assay urine, feces, and expired air collected from rats. Efforts should be made to identify any metabolite which comprises 5 percent or more of the administered dose and the major radioactive components of blood.

(*2*) **Changes in biotransformation.** Appropriate qualitative and quantitative assay methodology should be used to compare the composition of radioactive compounds in excreta from rats receiving a single oral dose (Groups B and C) with those in the excreta from rats receiving repeated oral doses (Group H).

(e) **Data and reporting.** The final test report should include the following:

(1) **Presentation of results.** Numerical data should be presented in tabular form. Pharmacokinetic data should also be presented in graphical form. Qualitative observations should also be reported.

(2) **Evaluation of results.** All quantitative results should be evaluated by an appropriate statistical method.

(3) **Reporting results.** In addition to the reporting requirements as specified in 40 CFR part 792, subpart J, the following specific information should be reported:

(i) Species and strains of laboratory animals.

(ii) Chemical characterization of the test substance, including:

(A) For the radioactive test substances, information on the site(s) and degree of radiolabeling, including type of label, specific activity, chemical purity, and radiochemical purity.

(B) For the nonradioactive compound, information on chemical purity.

(C) Results of chromatography.

(iii) A full description of the sensitivity, precision, and accuracy of all procedures used to generate the data.

(iv) Percent absorption of test substance after oral and dermal exposures to rats and dermal exposure to miniature pigs.

(v) Quantity and percent recovery of radioactivity in feces, urine, expired air, and blood. In dermal studies on rats and miniature pigs, include recovery data for skin, skin washings, and residual radioactivity in the covering as well as results of the washing efficacy study.

(vi) Tissue distribution reported as quantity of radioactivity in blood and in various tissues, including bone, brain, fat, gastrointestinal tract, gonads, heart, kidney, liver, lung, muscle, skin and in residual carcass of rats.

(vii) Materials balance developed from each study involving the assay of body tissues and excreta.

(viii) Biotransformation pathways and quantities of test substance and metabolites in excreta collected after administering single high and low doses to rats.

(ix) Biotransformation pathways and quantities of the test substance and metabolites in excreta collected after administering repeated low doses to rats.

(x) Pharmacokinetics models developed from the experimental data.